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Continuing Data as Claimed by Applicant

Foreign Applications

If Required, Foreign Filing License Granted 11/28/2000

**** SMALL ENTITY ****

Title

Velocity independent microfluidic flow cytometry

Preliminary Class

Data entry by : SMALLS, DONNA **Team :** OIPE **Date:** 02/08/2001

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PROVISIONAL

PATENT APPLICATION

Velocity Independent Microfluidic Flow Cytometry

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Velocity Independent Microfluidic Flow Cytometry

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DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention is directed to velocity independent flow cytometry. In particular, the present invention provides velocity independent microfluidic flow cytometry which allows measurement of relatively large materials, e.g., molecules including DNA's, peptides, and other polymers. Any microfluidic devices currently known to one of ordinary skill in the art can be used in the present invention. However, preferred microfluidic devices are constructed by single and multilayer soft lithography (MLSL) as described by Unger et al. in *Science*, **2000**, *288*, 113-116, and further detailed in commonly assigned U.S. Patent Serial Application No. 09/605,520, filed June 27, 2000, which are incorporated herein by reference in their entirety. Other preferred microfluidic devices are disclosed in a commonly assigned provisional patent application entitled "Microfluidic Devices and Methods of Use" (attorney docket no. 020174-002500US), which is filed even date with the present application.

Microfluidic flow cytometry for sorting cells and DNA's are disclosed in commonly assigned U.S. Patent Application Serial No. 09/325,667 and the corresponding published PCT Patent Application No. US99/13050, and U.S. Patent Application Serial No. 09/499,943, respectively, all of which are incorporated herein by reference in their entirety.

Current microfluidic flow cytometry provides fluorescence signal in which the area of the signal peak is velocity dependent. For example, as illustrated in Figure 1A, at a similar velocity the peak intensity and the peak area is proportional to the length of the DNA being detected by flow cytometry. However, as shown in Figure 1B, if two similar length of DNAs have different velocity, the faster moving DNA will have smaller peak area (in Figure 1B, the peak height has reached maximum which is indicated by the

dotted line) and the slower moving DNA will have larger peak area. This velocity difference may lead to misleading or erroneous interpretation flow cytometry data.

The present invention significantly reduces or eliminates the affect of molecule velocity through a detection zone on the peak area by eliminating or normalizing the velocity factor of materials. In particular, the present invention provides at least two different detection zones along the material's flow path to determine the velocity of each material which flows through the detection zones. By placing two different detection zones at a predetermined distance (i.e., "d") from each other, one can measure the velocity of a material flowing through the detection zones by measuring the time difference (i.e., "t") at which the material passes through the first detection zone and the second detection zone. Since the velocity (i.e., "v") is distance divided by time, the velocity of the material is calculated by the formula: $v=d/t$, where v, d and t are those defined above. The peak area is then divided by the velocity to eliminate the velocity factor. In this manner, a more accurate determination of the material size can be made.

While one can use multiple detectors and electromagnetic (e.g., laser for laser induced fluorescence) sources, it has been found by the present inventors that an acousto optic modulator in conjunction with an aperture is particularly suitable for providing two different detection zones from a single laser source. An acousto optic modulator is readily available from a variety of sources including at http://www.brimrose.com/acousto_modulators.html, which also includes a general discussion on the theory behind acousto optic modulator. Other devices which split the laser beam into two or more different positions can be used instead of an acousto optic modulator. Such devices are well known to one of ordinary skill in the art and include rotating mirrors, gratings and other electromagnetic wave diffracting devices. The aperture allows emission of only one particular diffracted beam to illuminate the detection zones and blocks other diffracted laser beam.

When using an acousto optic modulator, preferably the first order beam, which is typically about 20% intensity of the original laser beam entering the acousto optic modulator, is used. One particular embodiment of the present invention and its results are shown in Figures 2A and 2B, respectively. In this embodiment, the distance **10** between the two detection zones **14L** and **14R** is about 10 μm and the width of the fluid flow channel is about 5 μm. The laser beam enters an acousto optic modulator and the first order beam is emitted through an aperture (not shown). In this manner, only the

first order beam is used to illuminate both detection zones **14L** and **14R**. In order to scan the entire cross section of the fluid flow channel and to allow scan of two different detection zones **14L** and **14R**, the acousto optic modulator is adjusted such that the first order beam's x- and y-axis positions are allowed to vary at a particular frequency. In Figures 2A and 2B, the beam has y-axis frequency of 150 kHz, i.e., the beam travels from the "top" of the flow channel **20** to the "bottom" of the flow channel **24** at a rate of 150,000 times per second. In addition, it has a sampling rate of 40 kHz, i.e., each y-position is sampled about 4 times (150/40). Furthermore, the laser beam switches from the detection zone **14L** to **14R** and vice a versa at a rate of 5 kHz. Frequency of x-axis switching can be seen in the top graph of Figure 2B. In this graph, when the peak is at the top, it represents detection (or scanning) in the **14R** region, and when the peak is at the bottom (i.e., 0) it represents detection (or scanning) in the **14L** region. As can be seen, the laser beam moves from one position to another (in the x-axis) to allow scanning of two different positions. This allows a same material to be detected at two different times at two different regions as shown in the lower graph of Figure 2B. By determining the time difference between such detection and knowing the distance **10** (Figure 2A), one can calculate the velocity of the material traveling through the fluid flow channel. It should be appreciated that for more accurate determination, the solution should be dilute enough such that statistically only one material enters the detection zone at a time.

Thus, by normalizing the total peak area (from both **14L** and **14R** regions) in the bottom graph of Figure 2B one can plot a new normalized graph as shown in Figure 3. It has been found that the coefficient of variance in this particular reading is 5.7% which can be significantly reduced. By using 2.5 μm diameter beads and LIF, one can determine graph the time difference (which is approximately velocity^{-1}) vs. time as shown in Figure 4. As can be seen, there is a significant different in velocity of each materials. Velocity variation of materials occurs in all fluid flow systems; however, it is particularly pronounced in fluid pump systems that operate at a particular interval, which tend to create a discrete pulse of fluid flow rather than a continuous stream of fluid flow.

Gel-electrophoresis and other similar methods have limited resolution capacity for medium to large DNA molecules, and therefore are inapplicable in many cases. In contrast, methods of the present invention are not limited by the size of material (e.g., DNA). Moreover, if the distance between two detection regions are large or the velocity of the material is slow, one can use these variations to study a variety of

characteristics of the material. For example, one can detect changes in cells as it passes through from one detector to another. One can also analyze chromosome distribution in cells (e.g., karyotyping). Methods of the present invention are also useful in epidemiology and other diagnostic and assay procedures.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

WHAT IS CLAIMED IS:

1. A method for characterizing a material comprising the steps of passing said material through two different detection zones and characterizing said material based on its characteristics in both detection zones.

2. The method of Claim 1, wherein said method comprises velocity independent flow cytometry.

3. The method of Claim 1, wherein said material is characterized in a microfluidic device.

4. The method of Claim 3, wherein said method comprises a detection device comprising a laser beam generator, a laser beam splitter, and a means for detecting laser induced fluorescence.

5. The method of Claim 4, wherein said laser beam splitter is an acousto optic modulator.

ABSTRACT OF THE DISCLOSURE

The present invention is directed to velocity independent flow cytometry.

Single Molecule DNA Sizing in a Chip

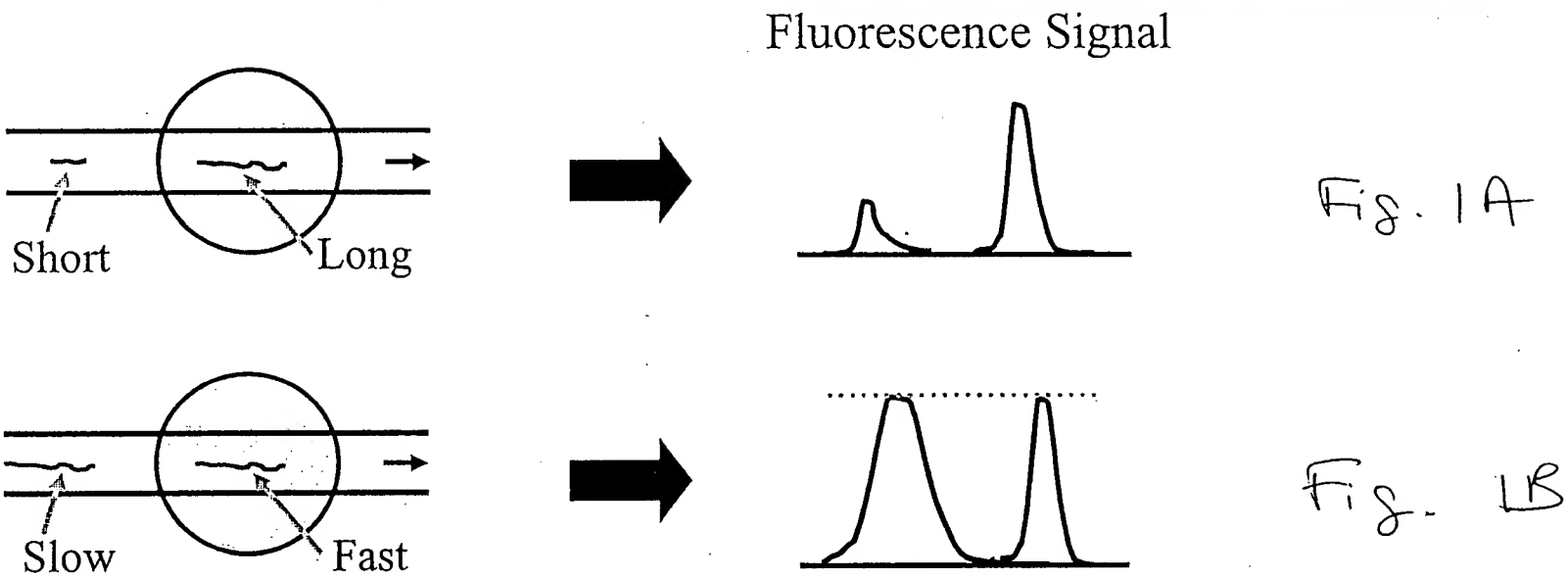


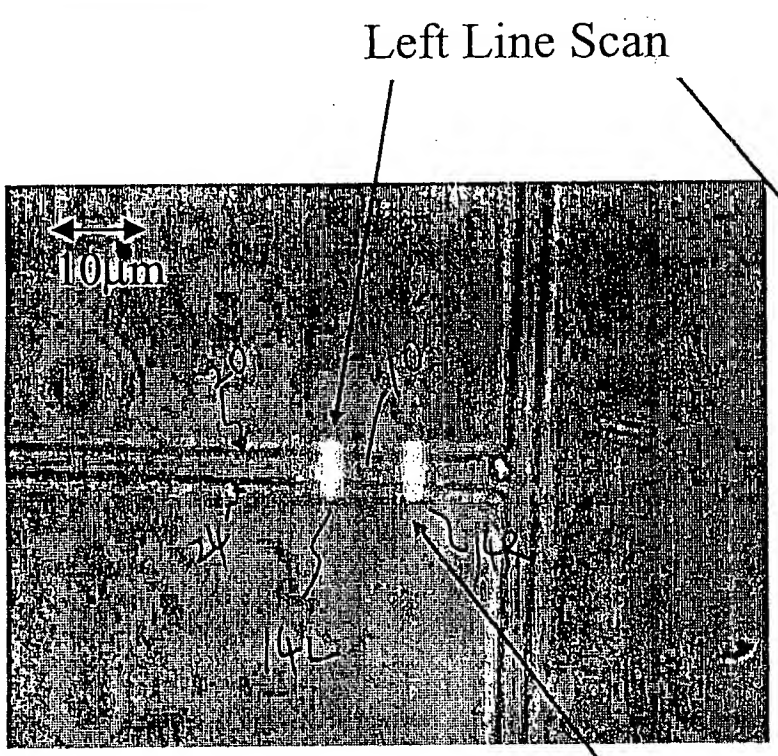
Fig. 1A

Fig. 1B

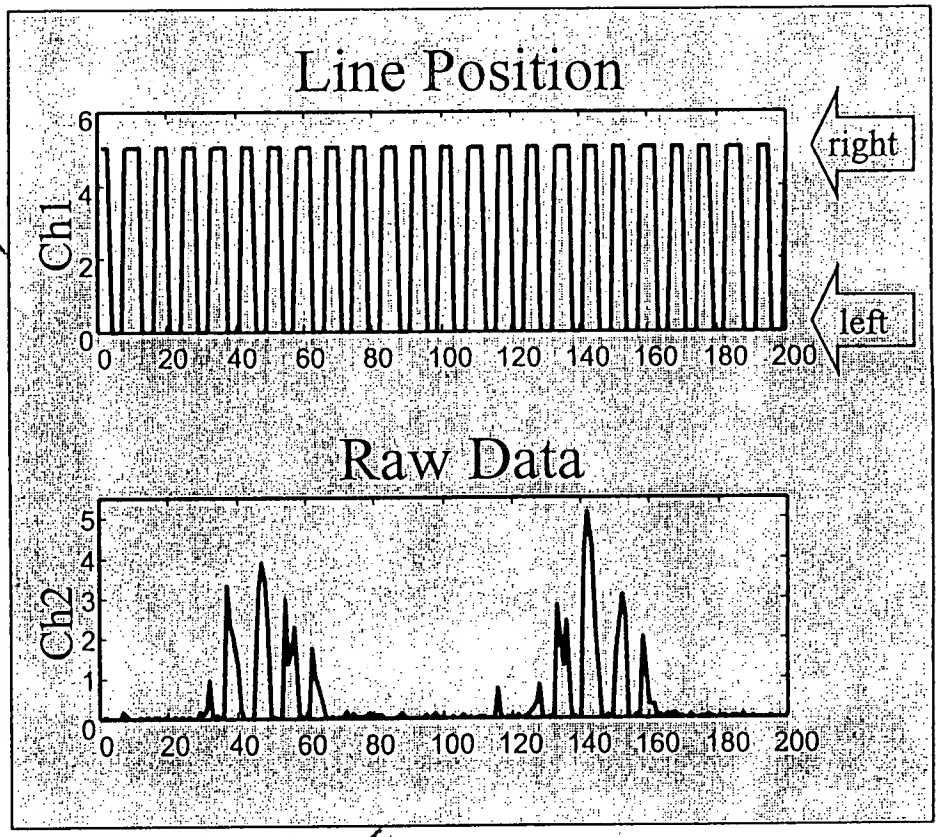
The area is velocity dependent

* Chou HP, Spence C, Scherer A, Quake S. A microfabricated device for sizing and sorting DNA molecules. Proc. Natl. Acad. Sci. USA 96:11-13 1999

VIM - *Velocity Independent Microfluidic Flow Cytometry*



Left Line Scan



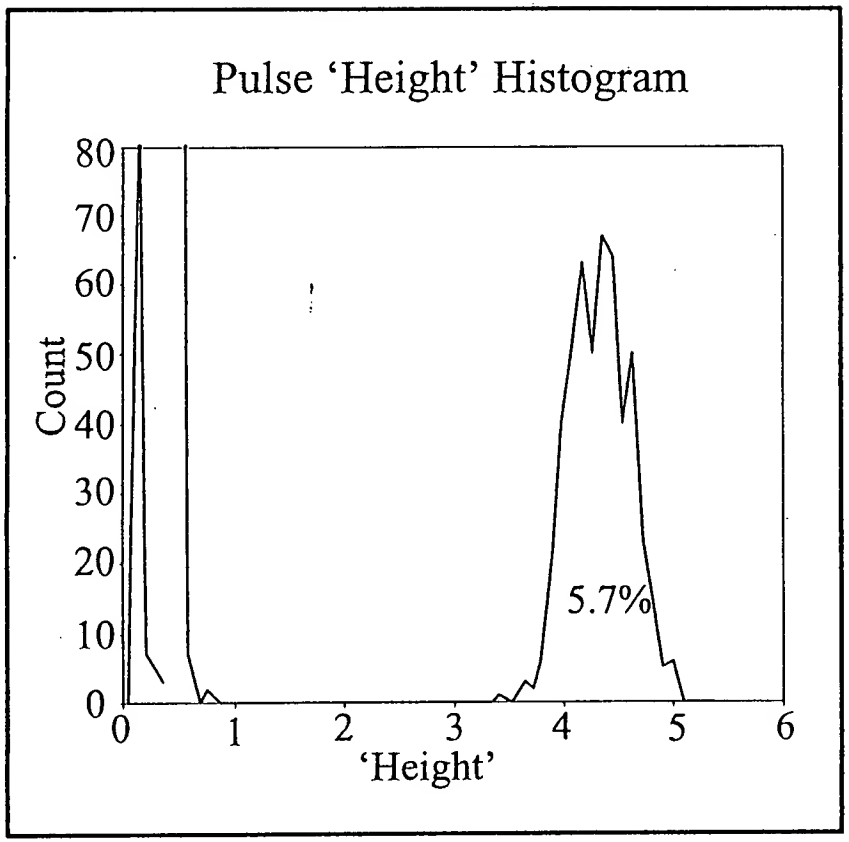
Right Line Scan

2D beam Scaning by
Acousto Optic Modulator

Fig 2A

Fig 2B

VIM - Preliminary Results: 2.5 m Beads



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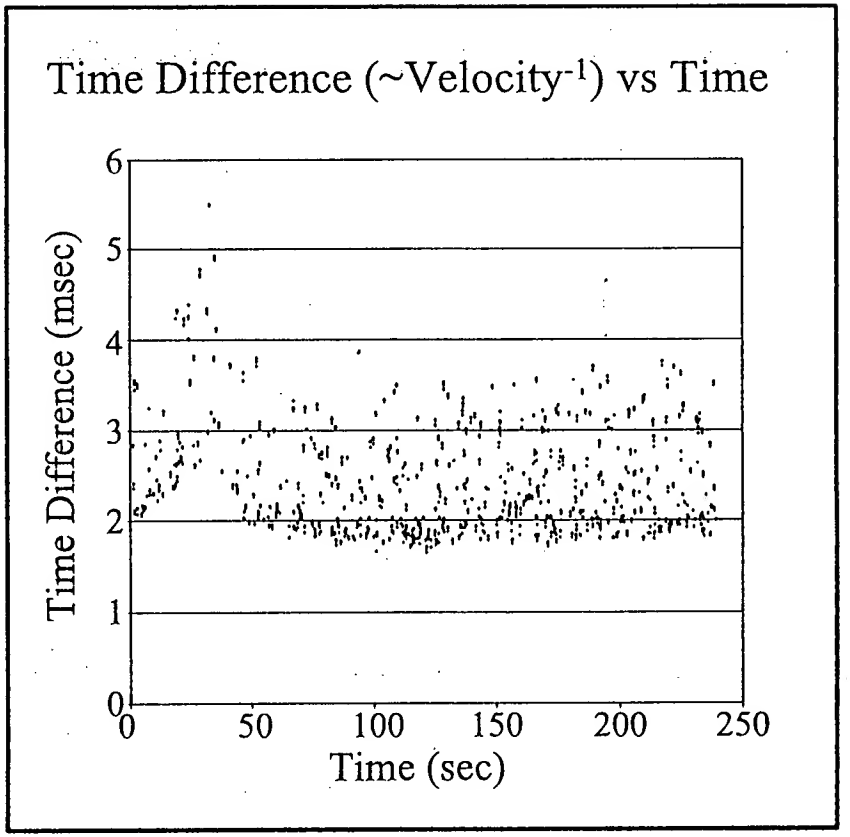

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